Collinear Optical Coherence Microscopy and Confocal Fluorescence Microscopy: A New In Vitro Imaging Method for Tissue Engineering

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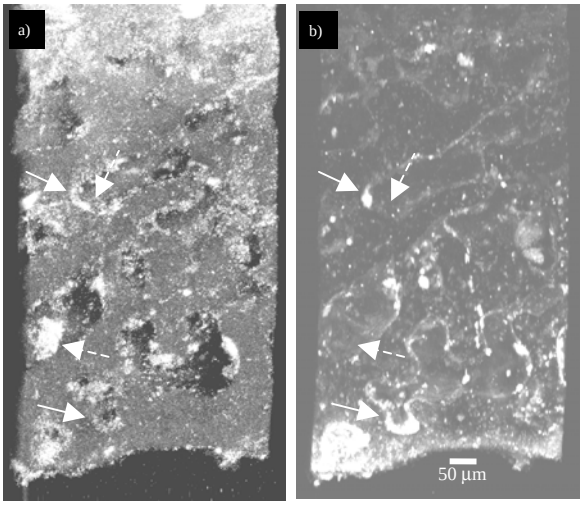
Tissue engineered medical products (TEMPs) often consist of a three-dimensional synthetic scaffold that provides form and foundation for the cells as they produce the tissue of interest. Successful TEMPs will allow cell infiltration, foster proliferation and differentiation within the scaffold. Cell infiltration and behavior may depend on a multitude of factors intrinsic to the scaffold, including global and local structure, surface composition, and other physical properties such as modulus. While it is generally understood that a complex interaction of many variables influences the success of the TEMPs, the precise nature of these interactions has yet to be worked out in many instances. A significant difficulty in further understanding the interaction between these factors and cell behavior is the lack of a high-resolution imaging technique that can penetrate deeply into the scaffold. Towards meeting this need, we have built a co-linear optical coherence and confocal fluorescence microscope (OCM/CFM) to non-invasively monitor both structure (OCM) and function (CFM) in a TEMP.

Laser scanning confocal microscopy has been routinely used to reveal details of cells, tissues, and their growth. However, the shape of its axial point spread function (PSF) results in relatively poor rejection of light scattered far from the focus and limits its use in highly scattering media such as TEMPs. OCM is an interferometric technique that combines both confocal and coherence gating mechanisms to improve stray light rejection. Thus, the PSF of OCM drops much more rapidly than that of confocal microscopy, making it much more sensitive. CFM has proven to be an extremely powerful technique for understanding cell viability, differentiation, and protein expression in tissue engineering. CFM, of course, suffers from the same relatively poor background rejection as reflection confocal microscopy. However, the added spectral discrimination can significantly extend the depth-of-view for the technique, provided that the background fluorescence is minimal.

In this work, we briefly describe OCM/CFM. We then use it to characterize polymer scaffolds seeded with osteoblasts and compare the images with those gathered using LSCM.



Volumetric renderings of 70 single-plane images (to 280 µm) obtained from a) OCM and b) CFM on a poly (ε-caprolactone) tissue scaffold containing stained osteoblasts and bone matrix. Dashed and solid arrows indicate examples of suspected areas of bone matrix and cell, respectively.